Sortase A

Week 15

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Transformation of Gibson Assembled pSB1C3-GB1-SortA into *E. coli* Top 10

Responsible

Aman Mebrahtu

Protocols used

- Transformation

Experimental Set Up

Two samples of Gibson assembled pSB1C3-GB1-SortA were transformed into chemically competent *E. coli* Top 10 cells.

Table 1: Transformation of pSB1C3-T7-Lys-LT

Sample	Comment
Gibson Assembly T1	Transformed into Top10 cells
Gibson Assembly T2	Transformed into Top10 cells

Results and Conclusions

No colonies were observed the next day. This indicated that either the Gibson Assembly or the transformation had been unsuccessful.

Discussion and Troubleshooting

Difficulties in achieving desired results from the Gibson assembly has led us back to using the overlap extension PCR method. In this strategy, we plan to first fuse the GB1 fragment to the sortase fragment by means of an overlap extension PCR. This fused fragment (GS) would then be digested on both ends with EcoR1 and Pst1 and later ligated into pSB1C3.

2 Overlap Extension PCR of GB1 and Sortase

Responsible

Reskandi Rudjito and Aman Mebrahtu

Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

The purpose of this PCR was to fuse together the GB1 sequence with truncated sortase sequence isolated from *S. aureus* . The sortase sequence has been designed to have a 20 bp GB1 overlap on the 5' end. This overlapping section served as a bridge to fuse the two sequences together. In the PCR reaction, primers were added to the 5' end of the GB1 and 3' on the sortase to amplify the fused fragment at the same time. The primers and PCR conditions used are written below.

- Forward primer: Prefix F
5'CACGAATTCGCGGCCGCTTCTAG'3
- Reverse primer: GS1 Suffix R
5'AAACTGCAGCGGCCGCTACTAGTATTAGTGGTGATGGTGATGATGTTTGACTTCTGTAG'3

Table 2: Overlap Extension PCR of GB1-Sortase

Reaction	Volume [μl]
5X Q5 Reaction Buffer	5
5X Q5 High GC Enhancer	5
Sterilized water	7.75
2 mM dNTPs	2.5
10 μlM Forward Primer	1.0
10 μlM Reverse Primer	1.0
GB1 (1 ng/μl	1.0
Sortase (1 ng/μl	1.0
Q5 High Fidelity DNA Polymerase	0.25

Table 3: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {[oC]}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		66/69	30 secs
Extension		72	20 secs
Final Extension	1	72	2 min
Hold	indefinitely	4	-

Table 4: Sample used for Overlap Extension PCR of GB1-Sortase

Sample	Comment
Sortase 1.2	Annealed at 66 and 69 oC
Sortase 2.1	Annealed at 66 and 69 oC
Sortase 2.2	Annealed at 66 and 69 oC

Results and Conclusions

The result of the overlap extension PCR is shown in the figure below. The expected size of the amplicon is 677 bp. Lane 2-4 shows results from an annealing condition of 66 oC. Whereas lane 5-7 shows the result at a higher annealing of 69 oC.

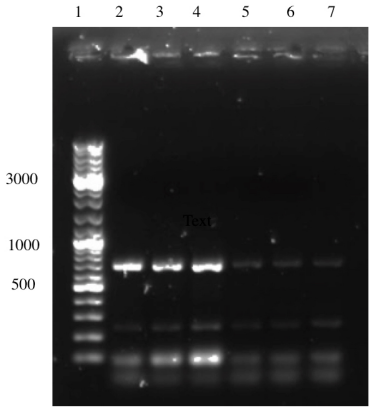

Figure 1: Overlap Extension PCR of GB1-Sortase. (1) Ladder (2) GB1-Sort 1.2 annealed at 66 oC (3) GB1-Sort 2.1 annealed at 66°C (4) GB1-Sort 2.1 annealed at 66°C (5) GB1-Sort 1.2 annealed at 69°C (5) GB1-Sort 1.2 annealed at 69°C (6) GB1-Sort 2.1 annealed at 69°C (7) GB1-Sort 2.1 annealed at 69°C

The PCR product from the samples annealed at 66°C were then subjected to gel purification. Figure 2 shows the gel used for this purification procedure.

Table 5: Concentration after gel purification

Sample	Concentration ng/μl
Sortase 1.2	5
Sortase 2.1	4
Sortase 2.2	sample was lost

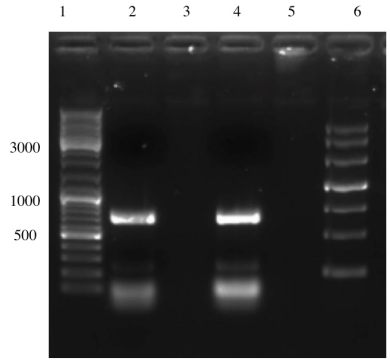

Figure 2: Gel purification of GS. (1) Ladder (2) GB1-Sort1.2 (3) Blank (4) GB1-Sort2.1 (5) Ladder

Discussion and Troubleshooting

Based on the results, we concluded that an annealing temperature of 69°C was too high and thus the fragment was not amplified efficiently.

3 Digestion and ligation of pSB1C3-GB1-SortA

Responsible

Reskandi Rudjito

Protocols used

- Digestion and Ligation

Experimental Set Up

To perform ligation procedure, the DNA fragments consisting of the Gb1-SortA, and pSB1C3 backbone were digested and purified prior to ligation. The concentrations of each DNA fragment are stated in the table below.

Table 6: Concentrations of DNA fragments for Ligation

Sample	Concentration [ng/μl]	Size [bp]
Digested Gb1-SortA (E and P)	5.0	677
Digested pSB1C3 (E and P)	5.7	2029

The insert:vector molar ratio used in the ligation was 10:1. The calculations shown on the tables below are adjusted so that each reaction contains 50 ng of backbone and the inserts are adjusted according to the desired molar ratio. The final reaction volume was 25 μl.

Table 7: Ligation Reaction of pSB1C3-T7-Lys-LT

Component	Volume [μl]
Gb1-Sortase 1.2	23
pSB1C3	8.77
T4 DNA Ligase	0.6
T4 DNA Ligase Buffer 10X	4.0
Sterile water	3.63

Results and Conclusions

The ligation product was transformed into TOP 10 Cells and one colony grew the next day. The ligation was successful.